Methods for the Isolation and Characterization of Constituents of Natural Products

I. Derivatives of Alcohols with Pyruvyl Chloride 2,6-Dinitrophenylhydrazone

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INTRODUCTION

This paper describes the preparation of a new acid chloride and esters from it with primary, secondary, and tertiary aliphatic alcohols. Development of the reagent was prompted by a need for a procedure for the direct isolation under mild conditions, of trace constituents in lipids which contain functional groups capable of reacting with acid chlorides. The acid chlorides described in the literature were found unsuitable to meet the objective either on structural grounds or by actual preparation and investigation of the properties of some of the more potential ones.

Van Duin (*1*) had prepared pyruvyl chloride 2,4-dinitrophenylhydrazone, but esters formed from this compound always existed in two isomeric forms. This reagent was, therefore, unsatisfactory for our purpose. The basic idea of using a dinitrophenylhydrazone of pyruvyl chloride, however, was considered sound. Preparation of a number of them resulted in the selection of one, namely, pyruvyl chloride 2,6-dinitrophenylhydrazone, which, in our hands, has proven to be a highly satisfactory reagent for derivatizing alcohols, amines, and mercaptans in lipids, the resultant derivatives being capable of direct isolation from the medium free of contamination.

APPARATUS AND REAGENTS

The following chemicals were used without further purification and were of the highest purity available from the commercial source specified:

2,6-dinitroaniline, cuprous chloride, and sodium nitrite were purchased from the J. T. Baker Co., Phillipsburg, New Jersey; pyruvic acid and hydrazine hydrate (85%) were obtained from the Fisher Scientific Co., Silver Spring, Maryland; 1,4-diazabicyclo (2.2.2.) octane (triethylenediamine) was obtained from Matheson, Coleman, and Bell Co., East Rutherford, New Jersey. Thionyl chloride (Fisher) was purified as described by Vogel (*2*). Benzene (Fisher) was purified by the method of Hornstein and Crowe (*3*), followed by treatment with basic alumina (Woelm) (*4*). Alumina, acidic (Woelm) purchased from Alupharm Chemicals, P.O. Box 20628, New Orleans, Louisiana, was weakened by the addition of 8% (v/w) distilled water, shaken until all lumps were broken, and permitted to stand overnight to equilibrate. The alcohols used in the study were obtained from various sources (Table 1) and were used without further purification. All primary alcohols were unbranched; all sec-

TABLE 1

PHYSICAL PROPERTIES OF ESTERS OF ALCOHOLS WITH 2,6-DNPH OF PYRUVYL CHLORIDE

Primary alcohols	Source[a]	m.p.[b]/C°	E[c]	C (%)		H (%)	
				Found	Calc.	Found	Calc.
C_1	F	175.5-177	5937	42.61	42.55	3.64	3.54
C_2	U.S.I.	171.5-172	5954	44.98	44.74	4.06	4.06
C_3	F	117-118	5951	46.61	46.60	4.42	4.53
C_4	F	91.5-92	5927	48.24	48.29	5.11	4.95
C_5	F	79-80	6003	50.02	49.85	5.49	5.34
C_6	E — K	67-68	6094	51.44	51.28	5.82	5.69
C_7	E — K	62-62.5	5913	52.95	52.60	6.02	6.02
C_8	F	55	5918	54.07	53.82	6.56	6.33
C_9	K + K	54-55	5987	55.10	54.90	6.81	6.61
C_{10}	E — K	63-63.5	5976	56.29	56.01	6.93	6.87
C_{11}	K + K	63.5	5957	57.29	57.00	7.42	7.12
C_{12}	B	72.5-73	5974	58.22	57.93	7.36	7.36
C_{13}	K + K	70.5-72	5955	58.98	58.79	7.49	7.57
C_{14}	B	77	5967	59.72	59.61	7.91	7.77
C_{15}	K + K	76	5987	60.43	60.37	8.10	7.96
C_{16}	B	83	5943	61.80	61.09	8.14	8.20
C_{17}	K + K	79.5-80	5923	62.50	61.78	8.47	8.31
C_{18}	B	87	5907	63.90	62.42	8.57	8.47
C_{19}	Columbia	83-83.5	5876	63.22	62.92	8.83	8.61
C_{20}	K + K	85-86	5863	65.46	63.40	8.93	8.74
C_{21}	K + K	85-85.5	5921		63.91	9.10	8.88
		Average	5959				

TABLE 1 (*Continued*)

Secondary alcohols (2-Hydroxy)	Source	m.p.	E	C (%) Found	C (%) Calc.	H (%) Found	H (%) Calc.
C_3	F	127.5-128	5932	46.86	46.60	4.63	4.53
C_4	E + K	118-119	6012	48.48	48.29	5.18	4.95
C_5	B	101.5-102	5898	50.40	49.85	5.41	5.34
C_6	F	63-64	5931	51.21	51.28	5.69	5.51
C_7	A	68-68.5	5854	52.84	52.60	6.31	6.02
C_8	E — K	36.5-37.5	6006	54.03	53.82	6.33	6.53
C_9	A	42-42.5	5874	54.69	54.90	6.42	6.61
C_{10}							
C_{11}	A	49-49.5	5965	56.95	57.00	7.12	7.17
C_{12}	A	41.5-43	5968	58.11	57.93	7.35	7.36
C_{13}	K + K	57-57.5	5932	58.93	58.79	7.36	7.48
C_{14}	A	53-53.5	5956	59.71	59.61	7.77	7.95
C_{15}	A	63.5-64.5	5963	60.13	60.37	7.96	7.75
C_{16}	A	58-60	5897	61.00	61.09	8.14	8.01
C_{17}	A	63-63.5	5952	61.82	61.78	8.31	8.54
C_{18}	K + K	57.5-58	5970	62.47	62.42	8.47	8.43
C_{19}	A	61-63	5909	63.04	62.92	8.83	8.79
		Average	5939				

TABLE 1 (*Continued*)

Tertiary alcohols	Source	m.p.	E	C (%) Found	C (%) Calc.	H (%) Found	H (%) Calc.
t-butyl	B	155-156.5	5811	48.55	48.29	5.19	4.95
t-amyl	F	105-106	5899	50.68	49.85	5.73	5.34
2-Me, 2-Pentyl	E — K	83.5-84.5	5935	51.30	51.28	5.94	5.68
3-Me, 3-Pentyl	E — K	119.5-120.5	5944	51.42	51.28	5.88	5.68
3-Me, 3-Hexyl	B	110-110.5	5881	52.61	52.60	5.87	6.02
3-Me, 3-Heptyl	B	77.5-78	5926	54.75	53.82	6.68	6.33
		Average	5893				

[a] Legend for sources: A = Aldrich Chemical Co., Milwaukee, Wisconsin; B = Baker Chemical Co., Phillipsburg, New Jersey; Columbia = Columbia Chemical Co., Columbia, South Carolina; E — K = Eastman-Kodak, Rochester, New Jersey; F = Fisher Scientific Co., Silver Spring, Maryland; K + K = K + K Labs, Plainview, New York; U.S.I. = U.S. Industrial Chem. Co., New York, New York.

[b] Melting points were obtained with the Fisher Johns apparatus and are uncorrected.

[c] Molar Extinction Coefficients were obtained in benzene at 400 mμ with a 1 cm^2 cell.

ondary alcohols were mono-2-hydroxy. The primary, n-C_{13} alcohol was prepared by the sodium borohydride reduction of methyl tridecanoate obtained from Applied Science Laboratories, State College, Pennsylvania.

EXPERIMENTAL

1-Chloro-2,6-dinitrobenzene was prepared from 2,6-dinitroaniline in approximately 80% yield by the method of Gunstone and Tucker (*5*).

2,6-Dinitrophenylhydrazine was prepared by a modification of the method of Borsche and Rantscheff (*6*). Ten g (0.05 moles) of 1-chloro-2,6-Dinitrobenzene is dissolved with the minimum of heat in 50 ml of 96% ethanol and an equimolar amount of hydrazine hydrate added in small portions through a reflux condensor so that the reaction does not become too vigorous. The hot solution is filtered at this point to remove traces of a black, insoluble impurity, refluxed for 15-30 minutes, and allowed to cool slowly to room temperature. The appearance of a dark, red crystalline material is indicative of the desired product; an orange-red impurity is sometimes present. The latter is water-soluble and is removed on the filter by washing the product with about 500 ml of warm water using suction. The yield of almost pure 2,6-dinitrophenylhydrazine is about 50%, m.p. 143-144°C (lit. 144-145°C (*6*)). The product is satisfactory for use without further purification.

Pyruvic acid 2,6-dinitrophenylhydrazone is prepared by suspending 2,6-dinitrophenylhydrazine in hot water (80°C, 6 ml/g) and adding an equilmolar amount of pyruvic acid. The mixture is stirred magnetically for an hour, cooled slowly to room temperature, and the bulky, yellow precipitate filtered off. The product is washed with cold (4°C) water and dried thoroughly. Yield 85-90%, m.p. 203-205°C. The product is used without further purification.

Found: 40.1% C; 2.98% H; 20.4% N
Theory: 40.3% C; 2.98% H; 20.8% N.

Pyruvyl chloride 2,6-dinitrophenylhydrazone is prepared by suspending one part (by weight) of pyruvic acid 2,6-dinitrophenylhydrazone in 2 parts (by volume) of thionyl chloride. The suspension is stirred magnetically and heated under reflux until clear. The solution is cooled slowly to room temperature, allowed to stand for 1 hour at 4°C, filtered with suction and dried *in vacuo* at room temperature until odorless. Yield about 70%, m.p. 156-158°C.

Found: 37.89% C; 2.43% H
Theory: 37.60% C; 2.52% H.

Esters of pyruvic acid 2,6-dinitrophenylhydrazone are prepared by dissolving (with heat) 23.5 mg (0.08 mmole) of the acid chloride per ml of purified benzene. Ten ml of this stock solution is pipetted into a dry flask and the alcohol added. Four to five drops of the alcohols containing 7 carbons or less are used; for alcohols containing more than 7 carbon atoms, 0.5 millimole is weighed out and transferred to the flask using purified benzene to effect transfer, if necessary. Five ml of a solution of triethylenediamine (containing 19.4 mg/ml of purified benzene) is added in 1 ml portions, shaking briefly between additions and the mixture allowed to stand at room temperature for 15-20 minutes. In the meantime a column of alumina is prepared by adding 15 g of the 8% acidic alumina to a chromatography tube (1.8 × 13 cm) containing a small wad of glass wool (pushed into the stem of the column to the juncture where the stem meets the tube) and half-filled with benzene. The excess benzene is permitted to drain and when just dry, the reaction mixture is poured carefully onto the column and collection of effluent is begun. When all of the reaction mixture has been added and drained, the sides of the tube are washed down with a few milliliters of benzene. After draining, benzene is added until all of the color is removed below the excess reagent band which remains at the top of the column. The effluent is evaporated on the steam bath under a gentle stream of nitrogen and the residue recrystallized from absolute alcohol. Yield, 75-95% depending on the purity of the parent alcohol.

The esters were recrystallized to constant melting point and to constant molar extinction coefficient. Usually only one recrystallization was necessary to obtain an analytically pure sample. The low-melting secondary alcohols usually required 2 or 3 recrystallizations.

RESULTS AND DISCUSSION

Table 1 summarizes some of the properties of the derivatives. In general the melting points are in a useful range and are relatively high as far as alcohols are concerned. The UV-visible spectra of the esters in benzene (determined with the Perkin-Elmer model 350 recording spectrophotometer) show one rather broad peak with maxima at 400-402 mμ, with no distinction apparent between the 3 classes of esters prepared thus far.

The esters are all bright yellow or yellow-orange in color. They are

stable in ordinary light and are readily soluble in most cold organic solvents with polarities between aliphatic hydrocarbons and the short-chain alcohols.

Tertiary alcohols form derivatives as readily as do the primary and secondary alcohols with the reagent. Triethylenediamine was found to be much superior to pyridine as a catalyst for the reaction. With pyridine as the catalyst much longer reaction times were necessary to obtain modest yields even at elevated temperatures. Details of this as well as the preparation of derivatives at the submicromole level will be subsequently published.

The mild conditions under which the derivatives are formed was one of the requisites set for the acid chloride to be of value in the isolation of alcohols from lipids. In addition to this the esters meet all of the other required characteristics such as high color, stability, and the capacity to ion-exchange under the proper conditions. They also possess the ability to adsorb strongly onto alkaline adsorbents. Furthermore, the esters chromatograph well and exist in only one isomeric form; they also may be separated into classes. Preliminary work indicates that the acid chloride will react quantitatively with all types of hydroxy compounds which are ordinarily encountered in lipids. Details on these aspects will also be subsequently published.

SUMMARY

Preparation of a new acid chloride, pyruvyl chloride 2,6-dinitrophenylhydrazone, and esters of it with primary, secondary and tertiary alcohols is described. The esters form readily in benzene solution in the presence of triethylenediamine in good yield within 20 minutes at room temperature. The derivatives are bright yellow to yellow-orange, have sharp and relatively high-melting points, and have an absorption maximum near 400 mμ in benzene. The esters have a molar extinction coefficient near 5950 in benzene. They possess properties which lend themselves well to direct isolation from lipids.

Reference to certain products or companies does not imply an endorsement by the Department over others not mentioned.

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